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Evaluation of Pathogenic Bacterial Contamination and Antimicrobial Activity of Some Liquid Herbal Medicinal Products Sold in Umuahia, Abia State, South-Eastern Nigeria

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Objectives: To evaluate pathogenic bacterial contamination and antibacterial activity of some liquid herbal medicinal products (LHMPs) sold on the streets of Umuahia, Abia State.

Method: Twenty LHMPs sold in Umuahia were screened for bacterial contamination. Isolation and identification were carried out using standard microbiological methods in Microbiology Department of Michael Okpara University between October and November 2015. The antimicrobial activity of the products that showed no bacteria growth (P3, P10, P13 and P17) were further evaluated by determining their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against some bacteria isolates. Antibiotics susceptibility pattern was evaluated using disc diffusion technique.

Result: Sixteen out of twenty LHMPs were contaminated. *Escherichia coli* was the highest isolate with 21.9% followed by *Staphylococcus aureus (*14.6%). *Salmonella* spp was the least occurring isolate with a percentage occurrence of 4.9%. Most isolates were resistant to amoxicillin and chloramphenicol. Levofloxacin and ceftriazone showed very encouraging results, with a percentage susceptibility of 100% and 88.9% in *E. coli* respectively. The highest total viable count among the products was 11.5 x 10^8cfu/ml while the least was 3.6 x 10^6 cfu/ml.

Conclusion: Most traditionally prepared liquid herbal drugs sold in Umuahia are grossly contaminated with a wide variety of potentially pathogenic bacteria.

Keywords*: Pathogenic bacteria, liquid herbal drugs, antibiogram*

Highlights:

- Eighty percent (80%) of the liquid herbal drugs examined were grossly contaminated by bacterial organisms.
- *Salmonella* spp which are known to cause gastro-enteritis were isolated from Liquid herbal products sampled in this study.
- Ceftriaxone and Levofloxacin showed a percentage susceptibility of 88.9% and 100% respectively to *E. coli* which was the most frequently isolated organism.
- Liquid herbal products which showed no growth on initial cultures demonstrated antimicrobial properties against Gram-negative and Gram-positive bacteria.

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Introduction

In most African countries including Nigeria, herbal medicine is recognized as an important component of health care system, especially among rural dwellers that constitute about 70% of the population.[1] A world health organization survey indicates that about 70-80% of the world population, particularly in developing nations; rely on non-conventional medicines mainly of herbal sources in their primary health care.[2] The presence of antibiotic resistant microbial isolates in the Herbal Medicinal Products (HMPs) could lead to transfer of antibiotic resistant traits to hitherto sensitivity gut or oral microflora of consumers.[3]

Approximately, 80% of the people in developing countries chiefly rely on herbal medicine for health care needs. One of the criticisms of liquid herbal medicine is lack of standardization and quality control profiles. The misclassification of species and the mistaken substitution is a real danger in the preparation and administration of liquid herbal medicine.[4]

Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments[5]. Phytochemical compounds are chemical substances formed during the plant's metabolic processes. These metabolites include: alkaloids, flavonoids, coumarins, glycoside, polysaccharides, phenols, tannin, terpenes and terpenoids[6]. The presence of these secondary metabolites in plants probably explains the various uses of plants for liquid herbal medicines.

Plant materials when crudely processed into medicinal concoctions usually carries a large number of microbes originating from the soil as microorganisms of various kinds are normally adhered to leaves, stems, flower, roots and seeds. Additional contaminants may also be introduced during harvesting, handling and production of various herbal remedies since no conscious efforts are made to decontaminate the herbs other than washing[7]. Herbal medicines are therefore vulnerable to contamination by microorganisms and as such are disposed to spoilage. Accordingly, gross microbial contamination of herbal medicinal products commonly consumed in Nigeria has been severally demonstrated.[1,8,9]

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Methods

Sample collection

Twenty samples of herbal drugs (all liquid) were purchased randomly from different outlets in Umuahia. The samples were within their shelf lives and stored at room temperature as indicated by their manufacturers.

Sample preparation

Ten test tubes each containing 9 ml of physiological saline were used. The herbal drugs were shaken properly and 1 ml was added to the first test tube followed by a 10 fold serial dilution. After which 1 ml was discarded from the last test tube making the final volume in all the test tubes 9ml.

Isolation and identification of pathogenic bacteria in liquid herbal drugs

A sterile calibrated (0.1 ml) wire loop was used to streak the liquid herbs onto blood, chocolate, mannitol salt and MacConkey agar plates respectively and incubated at 37°C for 18-24 hours. The resultant colonies were further purified and characterized using standard methods. Bacterial isolates were identified on the basis of their morphology and Gram stain reaction. Biochemical tests carried out include; catalase, coagulase, urease, indole, citrate, triple sugar iron, hydrogen sulphide, oxidase tests, methyl red and voges proskaur.

Sterility testing of Liquid herbal medicinal products (LHMPs)

Each sample was first filtered with a sterile mesh to remove debris. The samples were checked for purity by streaking on nutrient agar and incubated overnight. Samples that showed no bacteria growth were used for the study. The liquid medicinal herb samples were diluted with sterile distilled water to (%, v/v): 1.56, 3.125, 6.25, 12.5, 25 and 50 and undiluted liquid medicinal herbs (100.0%).

Preparation of inoculums

The inoculum was prepared by emulsifying overnight colonies from an agar medium on nutrient broth. A 0.5 McFarland standard was used for visual comparison to adjust the suspension to a density equivalent to approximately 10^8 CFU/mL. Media plates were inoculated within 30 minutes of standardizing the inoculum, to avoid changes in inoculum density.

Antimicrobial activity of liquid medicinal herbs

The agar disc diffusion method on Muller-Hinton agar was used to determine the antibacterial

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activity of selected antibiotics against the bacterial isolates [10]. Fifteen millimeter of sterile Muller-Hinton agar was dispensed into sterile Petri dishes and allowed to solidify. A micropipette was used to introduce 100 µl of the inoculum onto the agar plate, and spread with glass rod spreader under sterile conditions. The plates were allowed to dry for 3-5 minutes. Whatman No 1 filter paper discs (Whatman International Ltd., UK), 6 mm in diameter were used to prepare the liquid herbal drug discs. Whatman No 1 filter paper discs were sterilized with an autoclave and soaked in the different liquid medicinal herbs dilutions, and dried at room temperature. Thereafter, all discs were placed on the plates pressed gently to ensure complete contact with the agar. A distance of at least 15 mm was maintained from the edges of the plates to prevent overlapping of inhibition zones. These processes were repeated for each liquid medicinal herb samples and for each of the bacterial isolates which were then incubated at 37°C for 24 hours. After incubation, the inhibition zone diameters produced by the different concentrations of the liquid medicinal herbs were measured using a transparent meter rule in millimeter. Diameter zones of inhibition were then interpreted using the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[12]

Determination of minimum inhibitory concentrations (MICs) of liquid medicinal herbs

Determination of the MIC was carried out as described by Gutpe[11] with slight modifications. The minimum inhibitory concentration of the extracts was determined for the test organisms in triplicates at varying concentrations of 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 (%, v/v). To obtain these concentrations, 1.0 ml of varying concentrations of the liquid medicinal herbs containing double strength of the concentrations (100, 50, 25, 12.5, 6.25, 3.125 and 1.56 (%, v/v)) were constituted in different test tubes, 1.0 ml of Muller-Hinton broth was added and then a loopful of the test organism, previously diluted to 0.5 McFarland turbidity standard, was introduced. A control was set up which contains Mueller-Hinton broth only without the liquid medicinal herbs. All the bacterial cultures were incubated at 37 ± 2°C for 24 hours. After incubation, each tube was examined for microbial growth by observing for turbidity. Hence any tube that did not show growth was an indicator that the test organism was inhibited by the liquid medicinal herb. The lowest concentration of the liquid medicinal herbs that inhibited the growth of the test organisms as detected by lack of visual turbidity was designated the minimum inhibitory concentration (MIC).

Determination of minimum bactericidal concentrations (MBCs) of the liquid medicinal herbs

MBC was determined by selecting tubes that showed no bacterial growth during the MIC determination. A loopful from each of the tubes was subcultured on the Mueller Hinton Agar. The plates were incubated for 24 hours at 37°C ± 2°C. The MBC was determined as the least concentration that showed no visible growth on the plate.[10]

Antibiotic sensitivity test with standard antibiotics

The bacteria isolates from the samples were reactivated by sub-culturing from slants agar unto nutrient agar plate and was incubated for 18-24 hours. The standardized inocula were swabbed on a nutrient agar plate. The optune disc (Nigeria) was used. The discs were placed on inoculated plates and pressed firmly unto the agar plate for complete contact. The bacteria strains were tested against the following disc; Ciprofloxacin (CPX, 10µg), Norfloxacin (NB, 10µg), Gentamicin (CN, 10µg), Amoxil (Am, 20µg), Streptomycin (S, 30µg), Rifampicin (RD, 20µg), Chloramphenicol (CH, 30µg) and Levofloxacin (LEV, 20µg). The plates were incubated at 37°C for 18-24 hours. Susceptibility of the isolates to the antibiotics was shown by a clear zone of inhibition which was measured in millimeters with a transparent meter. The diameter zone of inhibition obtained was then compared with the clinical and laboratory (CLSI) standard.[10]

Results

Table 1 shows physical characteristics, components and bacteria isolated from various liquid herbal medicines. All the information was obtained from the manufacturer's leaflet while the prevalence and mean bacterial load of various isolates from liquid herbs analyzed are shown in Table 2.

Table 3 shows the total viable count. P19 showed the highest total viable count and P16 the lowest total viable count while four (4) different liquid herbs showed no bacterial growth.

Table 4 shows the antibiotic susceptibility profile of the bacterial isolates from liquid herbal drugs in Umuahia. Amoxyl and chloramphenicol were least susceptible to most isolates while the fluoroquinolones, ceftriazone and gentamicin

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showed highest susceptibility to the bacterial isolates.

Table 5 shows percentage antibacterial activity of liquid medicinal herbs. P3, P10, P13 and P17 showed no antibacterial activity against the test organisms (*E. coli*, *Klesiella* spp, *S. aureus* and *Proteus* spp which were isolated from the contaminated medicinal herbs) at concentrations ranging from 100 to 25 % (v/v) while it showed different antibacterial activity at concentrations between 3.125 and 1.56% (v/v).

Table 6 shows the MIC and MBC of liquid medicinal herbs with antibacterial activity. Results showed that MIC and MBC values for the liquid medicinal herbs ranged between 3.125% and 50%.

Table 1: Physical characteristics, components and bacteria isolated from various liquid herbal medicines

Drug	Shelf Life	Colour	Odour	Composition	Organisms isolated	Therapeutic claim
P1	3 years	Brown	Ginger-like smell, non-offensive	*Rhamus purshia* (3kg) *Tamaridus indica* (4kg) *Alium sativum* (1kg) Linum (3kg) *Zingiber officinale* (3kg) *Fistlula* (3kg)	*Bacillus subtilis* (++)	Infections, Staphylococcus, Builds immune system, Antiviral.
P2	2 years	Brown	Non-offensive colour	*Alium sativum* (3w/w) Xylopia aromatic (5w/w) *Tetrapleur tetraptra* (7w/w) Ficuscarica (10w/w) Nauclealatifolia (25w/w) *Streculia urens* (151w/w) Combretummiscranthm (35w/w) Water	*Staphylococcus aureus* (++) *Proteus mirabilis* (++) Coagulase Negative *Staphylococcus* (++)	Measles, Skin rashes, Chicken pox, Small pox.
P3	3 years	Dark	Juicy smell, almost odourless	*Vernonia amygdalina* (12%) *Saccharum officinarum* (11.5%) *Allium sativum* (13%), *Cajanus cajan* (11.5%) Caramel (1.5%) *Zingiber officinale* (0.5%)	No growth	Cures piles, Purifies blood, Softens hard stool, Eliminates internal heat, Cures infection, Increases sexual libido.
P4	1 year	Light almost colourless	No odour	Lymbopogin citrates Indica bark *Treculia africana* *Allium sativum* *Zingiber officinale* root *Papaya* leaves	*Proteus mirabilis* (++) *Salmonella* spp(+) *Escherichia coli* (+++)	Reduces sugar level and cholesterol
P5	6 months	Cream	No odour	*Allium sativum* *Naudeadiiderchi* *Cochiospermum planchoni* *UvariachameI* *dippocratapalens*	*Proteus mirabilis* (+) Coagulase Negative *Staphylococcus* (++) *Escherichia coli* (++)	Prevents and treats malaria
P6	6 months	Light brown	Non-offensive odour	*Dogoyaro* leaves *Carica papaya* leaves, *Mangifera indica* leaves Water	*Proteus vulgaris*(+) *Pseudomonas aeruginosa*(+) *Klebsiella* spp(+++) *Staphylococcus aureus* (++)	Cures malaria, Anti bacterial.

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P7	6 months	Cream/ White	Ground maize like smell, non-offensive	Natural roots and barks Lemon grass *Aloe barbaders*	*Escherichia coli* (+++) *Bacillus spp*(++) *Pseudomonas aeruginosa*(+)	Anti bacterial, Cures typhoids.
P8	6 months	Light brown	Rotten smell	Natural roots and barks Leaves Water Garlic	*Pseudomonas aeruginosa*(++) *Escherichia coli* (+++) *Streptococcus* spp(+) Coagulase Negative *Staphylococcus* (+)	Painful menstruation, Irregular menstruation.
P9	3 months	Colourless	Offensive rotten smell	*Aloe vera* Lemon grass Flowers and natural roots Water	*Escherichia coli* (+++) *Staphylococcus aureus* (++) *Pseudomonas aeruginosa*(++)	Increases sexual libido, cures weak erection.
P10	2 years	Dark	Non offensive odour	*Vernonia amygdalina* *Saccharum officinarum* *Lymbopogin* citrates Indica bark *Treculia africana* *Allium sativum, Cajanus cajan* Caramel *Zingiber officinale*	No growth	Increases sexual libido, Prevents toilet infection, Purifies the blood.
P11	2 years	Army green	Offensive	Garlic, Water, 9 indian roots	*Staphylococcus aureus* (+) *Escherichia coli* (++) *Proteus vulgaris*(++)	Immunity booster, Painful menstruation, Gonorrhoea, Waist pain, Malaria and typhoid, Intestinal diseases, Vaginal itching, Vaginal discharge, Syphilis.
P12	3 years	Light brown	Offensive	Babama (15%) Water (10%) Conto (15%) Suite (10%)	*Streptococcus* spp(+) *Klebsiella* spp(+++) *Bacillus spp*(++) *Staphylococcus aureus* (++)	Cures teeth pain, gum decay, mouth odour, scurvy.
P13	3 years	Pink	Soapy like smell	*Aloe vera* 10.9, Honey 0.5 Jakpuline 9.5, Water 0.5, Colour 0.1	No growth	After shave cream, Rashes, Ringworm, Pimples, Scabies, Foot rot, Eczema, Chicken pox,
P14	3 months	Light almost colourless	Sweet smell	Natural roots	*Escherichia coli* (+++) *Bacillus spp*(++) *Streptococcus* spp(++) *Staphylococcus aureus* (++)	All facial infections, eye problem.
P15	3 months	Almost colourle	Offensive odour	Bread fruit bark Citrus lemon leaves	*Proteus vulgaris*	Waist pain.

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		ss		Ginger roots *Cymbopogon spp* *Carica papaya* *Psidium guajava*	(+) *Escherichia coli* (++)	
P16	2 years	Colourless	Odourless	Different types of roots, barks and leaves	*Escherichia coli* (+++)	Heals and strengthens the bone.
P17	1 year	Light wine colour	Fermented palmwine like smell	Natural roots and herbs	No growth	Heals fractures, provides calcium to strengthen bone.
P18	3 years	Black	Sweet smell	Palm kernel nuts	*Escherichia coli* (+++)	A probiotic cures cough.
P19	1 year	Dark	Offensive odour	*Alium sativum* *Aloe vera* Lemon grass Uda roots	*Streptococci* spp(++)	Cures toilet infection.
P20	3 years	Ash	Sweet smell	Nihomas-HNY LL-YP Taspen-LM Damez-CB	*Klebsiella* spp(+++) *Bacillus* spp(++) *Salmonella* spp(++)	Typhoid.

KEY : + = Scanty growth (1-20 colonies), ++ = Moderate growth (21-50 colonies), +++ = Profuse growth (≥ 51 colonies).

Table 2: Prevalence and mean bacterial load of various isolates from liquid herbs analysed

Bacterial Isolate	*Frequency (%)*	*Number of occurrence*	*Mean bacteria load*
E. coli	21.9%	9	$3.5 \pm 5.48 \times 10^6$
Klebsiella spp	7.3%	3	$2.5 \pm 3.00 \times 10^6$
S. aureus	14.6%	6	$3.0 \pm 6.37 \times 10^6$
P. aeruginosa	7.3%	3	$1.6 \pm 4.73 \times 10^6$
Streptococcus spp	9.8%	4	$1.0 \pm 3.56 \times 10^6$
Bacillus spp	12.2%	5	$1.7 \pm 3.97 \times 10^6$
Salmonella spp	4.9%	2	$8.0 \pm 2.83 \times 10^5$
Proteus vulgaris	7.3%	3	$1.8 \pm 2.52 \times 10^6$
Proteus mirabillis	7.3%	3	$7.0 \pm 2.52 \times 10^5$
CoaNS	7.3%	3	$6.0 \pm 2.00 \times 10^5$
Total	*100.00%*	*41*	

CoaNS = Coagulase negative Staphylococcus

Table 3: Total Viable Count

S/N	*Liquid herbal drugs code*	*Total Viable Count*
1.	P1	1.0×10^7
2.	P2	4.5×10^6
3.	P3	No growth
4.	P4	9.0×10^6
5.	P5	5.0×10^6
6.	P6	1.0×10^7
7.	P7	4.0×10^6
8.	P8	6.0×10^6
9.	P9	7.0×10^6
10.	P10	No growth
11.	P11	4.0×10^6
12.	P12	6.0×10^6

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13.	P13	No growth
14.	P14	6.3×10^6
15.	P15	5.5×10^6
16.	P16	3.0×10^6
17.	P17	No growth
18.	P18	6.0×10^6
19.	P19	11.5×10^8
20.	P20	7.0×10^6

Table 4: Antibiotic susceptibility profile of the bacterial isolates from liquid herbal drugs in Umuahia

Bacterial Isolate	*No tested*	*Number of organism (%) sensitive to*								
		CPX	*NB*	*CN*	*AML*	*S*	*RD*	*CH*	*CRO*	*LEV*
E. coli	9	8(88.9)	3(33.3)	7(77.8)	1(11.1)	6(66.7)	4(44.4)	1(11.1)	8(88.9)	9(100)
Klebsiella spp	3	2(66.7)	1(33.3)	2(66.7)	0(00)	2(66.7)	1(33.3)	0(00)	2(66.7)	3(100)
S. aureus	6	4(66.7)	2(33.3)	4(66.7)	0(0)	2(33.3)	2(33.3)	0(0)	5(83.3)	4(66.7)
P. aeruginosa	3	2(66.7)	0(00)	3(100)	0(0)	1(33.3)	0(00)	0(0)	2(66.7)	2(66.7)
Streptococcus spp	4	2(50)	2(50)	1(25)	0(0)	1(25)	0(0)	0(0)	1(25)	4(100)
Bacillus spp	5	2(40.0)	0(0)	5(100)	0(0)	0(0)	0(0)	1(25)	4(100)	3(75)
Salmonella spp	2	2(100)	2(100)	2(100)	1(50)	1(50)	2(100)	0(0)	2(100)	2(100)
Proteus mirabilis	3	2(66.7)	2(66.7)	2(66.7)	3(100)	0(0)	2(66.7)	1(33.3)	3(100)	3(100)
Proteus vulgaris	3	3(100)	2(66.7)	2(66.7)	1(33.3)	1(33.3)	0(0)	2(66.7)	3(100)	3(100)
CoaNS	3	2(66.7)	0(00)	1(33.3)	0(00)	1(33.3)	2(66.7)	0(0)	1(33.3)	2(66.7)

Keys: CPX= Ciproflax, NB= Norfloxacin, CN= Gentamicin, AML= Amoxyl, S= Streptomicin, RD= Rifampicin, CH= Chloramphenicol, LEV= Levofloxacin, CRO= Ceftriazone, CoaNS= Coagulase Negative *Staphylococcus*.

Table 5: Percentage antibacterial activity of liquid medicinal herbs

Herb	*Organism*	*100%*	*50%*	*25%*	*12.5%*	*6.25*	*3.125%*	*1.56%*
P3	*E.coli*	-	-	-	-	+	+	+
	Klebsiella spp	-	-	-	-	-	+	+
	S. aureus	-	-	-	-	-	+	+
	Proteus spp	-	-	-	-	-	+	+
P10	*E.coli*	-	-	-	-	-	+	+
	Klebsiella spp	-	-	-	-	+	+	+
	S. aureus	-	-	-	-	-	+	+
	Proteus spp	-	-	-	-	+	+	+
P3	*E.coli*	-	-	-	+	+	+	+
	Klebsiella spp	-	-	-	-	-	+	+
	S. aureus	-	-	-	-	+	+	+
	Proteus spp	-	-	-	+	+	+	+
P17	*E.coli*	-	-	-	-	-	+	+
	Klebsiella spp	-	-	-	+	+	+	+
	S. aureus	-	-	+	+	+	+	+

\+ = Growth
\- = No growth

Discussion

The evaluation of liquid herbal drugs for the presence of pathogenic bacteria is expected to be regular due to the inevitable contamination of these products arising from their sources and preparations. Since they are readily available and relatively cheap, it has almost become the preferred

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treatment option especially in the tropics and common among the rural dwellers.

The study showed that the mean bacterial counts of the preparations were higher than the accepted values for non-sterile pharmaceutical products. This observation agrees with the reports of other researchers.[1,13] The presence of microbial contaminants in non-sterile pharmaceutical products can reduce or even inactivate the therapeutic activity of the products and has the potential of adversely affecting patients taking the medicine.[14] Some of these heavily contaminated preparations have caused severe gastrointestinal symptoms in some patients.

In the present study, both Gram-positive and Gram-negative bacteria were commonly isolated. It agrees with the reports of other researchers.[15,16] The most frequently isolated organisms in the study were *E. coli* (21.9%), followed by *S. aureus* (14.6%). This finding was at variance with reports of Okunlola et al., 2007[13] and Ujam et al., 2013.[15] Both authors reported *S. aureus* as the most frequently isolated followed by *E. coli*. *E. coli* is an intestinal bacterium and indicates faecal contamination; its presences in the herbal preparation showed poor hygiene and lack of proper handling of the products. *S. aureus* commonly observed in these reports above may be from human skin and anterior nares.

The findings that *Bacillus* spp were the third most frequently isolated bacteria in this study compares favourably with the reports of Ujam *et al*[15]. *Bacillus* spp are widely distributed in the soil, dust and air and since their spores are resistant to environmental destructive factors, they commonly contaminate surfaces and medications. Although *Bacillus* spp are regarded as non-pathogenic, some reports have described serious human infections caused by these organisms.[17,18] The presence of other members of the enterobacteraceae such as *Proteus* spp, *Klebsiella* spp and other bacteria such as *Pseudomonas aeruginosa* and *Streptococcus* spp and Coagulase negative *Staphylococcus* in the present study was also reported by WHO.[2] Although the pathogenicity of these organisms was not assessed, species of these agents have been incriminated in serious human infections.[19] The presence of *Salmonella* spp isolated from herbal products in this study portend a great danger for patients who consume some of the herbal products. This organism would cause gastroenteritis when ingested at very low doses. This agrees with the reports of Ujam *et al*who also observed *Salmonella* spp in their research.[15]

The antibiotic susceptibility of most of the isolates against the fluoroquinolones and the aminoglycosides was quite encouraging and compares favorably with the reports of Esimone et al.[16] Ceftriazone and Levofloxacin showed a percentage susceptibility of 88.9% and 100% respectively to *E. coli*. This finding agrees with the reports of Ujam et al[15] and Esimone et al., 2007.[16] The presence of resistant organisms in contaminated liquid herbal medicinal products is of public health importance. Resistance genes are found on mobilizable genetic elements called transposons. The ability of transposons to integrate into either conjugative plasmids or the organism's chromosome enhances the transferability of a given resistant determinant.[20] There are constant treatment failures due to acquisition of resistance by microorganisms. The mean bacterial count obtained in the present study for each isolate was quite higher than what was reported by Okunlola et al.[13] The MIC and MBC obtained from the study when the sterile products were treated with standardized test isolates demonstrated antimicrobial properties against Gram-negative and Gram-positive bacteria. Such liquid herbs can be harnessed for the treatment of infections in view of the ever increasing development of resistance against conventional antibiotics.

Table 6: Minimum inhibitory and bactericidal concentration of liquid medicinal herbs with antibacterial activity

Herb	*Organism*	*MIC (%)*	*MBC (%)*
P3	*E. coli*	12.5	25
	Klebsiella spp	6.25	12.5
	S. aureus	6.25	12.5
	Proteus spp	12.5	25
P10	*E. coli*	3.125	6.25
	Klebsiella spp	12.5	25
	S. aureus	6.25	12.5
	Proteus spp	12.5	25
P13	*E. coli*	25	50
	Klebsiella spp	12.5	25
	S. aureus	6.25	12.5
	Proteus spp	25	50
P17	*E. coli*	6.25	12.5
	Klebsiella spp	25	50
	S. aureus	NT	NT
	Proteus spp	NT	NT

MIC (%) = Percentage Minimum Inhibitory Concentration
MBC (%) = Minimum bactericidal Concentration
NT = Not Tested

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Conclusion

This study showed that most of the liquid herbal drugs examined (80%) were grossly contaminated by bacterial organisms. Ceftriazone showed a percentage susceptibility of 88.9% and 100% respectively to *E. coli* which was the most frequently isolated organism. Increased effort to develop suitable standardization method for preparations and constant monitoring of microbial standard of liquid herbal medicine should be ensured.

Conflict of interest

There is no conflict of interest.

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